**ACCELERATED EMERGENCY USE AUTHORIZATION (EUA) SUMMARY**
**UDX SARS-COV-2 MOLECULAR ASSAY**
**ULTIMATE DX CORP (UDX LABORATORIES)**

For *In vitro* Diagnostic Use
Rx Only
For use under Emergency Use Authorization (EUA) only

**(The UDX SARS-CoV-2 Molecular Assay will be performed at Ultimate Dx Laboratory, certified under the Clinical Laboratory Improvement Amendments of 1988(CLIA), 42 U.S.C. §263a, as per the Instructions of Use that were reviewed by the FDA under this EUA.)**

## INTENDED USE

The UDX SARS-CoV-2 Molecular Assay is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 RNA in upper respiratory specimens (such as nasal swabs, nasopharyngeal swabs, oropharyngeal swabs) from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to the Ultimate Dx Laboratory, which is a Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, certified high-complexity laboratory.

Results are for the detection and identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infective status. Positive results do not rule out bacterial co-infection with other viruses. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The UDX SARS-CoV-2 Molecular Assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The UDX SARS-CoV-2 Molecular Assay is only for use under the Food and Drug Administration's Emergency Use Authorization.

## DEVICE DESCRIPTION AND TEST PRINCIPLE

The UDX SARS-CoV-2 Molecular Assay is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set is designed to detect SARS-CoV-2 RNA in upper respiratory samples of patients suspected of COVID-19 by their healthcare provider.

The test is performed using the KingFisher Flex Purification System for RNA extraction and the Applied Biosystems QuantStudio 12K platform System for reverse transcription, PCR amplification and detection.

The UDX SARS-CoV-2 Molecular Assay includes: a Reaction Mix comprised of the PCR primers and probes for the detection of ORF1ab SARS-CoV-2 RNA and the β-actin endogenous control, an Enzyme Mix containing Uracil DNA Glycosylase, reverse transcriptase and *Taq* DNA polymerase, a Positive Control comprised of MS2-phage-based pseudo-virus containing the SARS-CoV-2 target region and a Negative Control comprised of DNase/RNase-free water.

**Table 1: Description of Gene Target Fluorescent Dyes**

| Contents | Assay | Dye |
|---|---|---|
| UDX 2019-nCoV Assays Multiplex | SARS-CoV-2 ORF1ab conserved region | 6-FAM |
| | IPC (human beta actin) | VIC/HEX |

## INSTRUMENTS USED WITH TEST

The UDX SARS-CoV-2 Molecular Assay is to be used with the following PCR instruments:

- Applied Biosystems QuantStudio 12K platform (QuantStudio 12K Flex Software v1.4)

The test is intended to be used with the automated extraction procedure KingFisher Flex Purification System (ThermoFisher Scientific, Cat#501527924).

## REAGENTS AND MATERIALS

The UDX SARS-CoV-2 Molecular Assay has been validated using only the components referenced in this submission.

**Table 2: UDX SARS-CoV-2 Molecular Assay Reagents**

| Reagent | Manufacturer | Catalog # |
|---|---|---|
| UltimateDx SARS-CoV-2 Assay | BGI (BGI Biotechnology, Wuhan Co.,Ltd) | HW5105 |
| KingFisher Flex Purification System | ThermoFisher Scientific | 501527924 |

## CONTROLS TO BE USED WITH THE UDX SARS-COV-2

Controls that provided with the test kit are as follows:

- A Negative Control ("No Template" control) comprised of DNase/RNase-free water and will be used to check the integrity of the entire procedure and will be used in each run. This control has no template DNA and therefore should not yield any amplification signal.
- A Positive Control: Comprised of a mixture of non-infectious MS2-phage-based pseudo-virus containing the SARS-CoV-2 RNA target sequence ($10^4$ copies/mL) and human β-actin RNA transcripts ($10^5$ copies/mL). The SARS-CoV-2 target sequence is packed in MS2-phage envelope protein as a surrogate for native virus. Positive control for this assay and will be included in each run to monitor entire procedure including extraction and amplification.
- Internal Positive Control (IPC): Human beta actin is used as an exogenous internal positive control. This control will undergo real-time RT-PCR independent of Cov2 targets and is used to verifying sample adequacy as its amplification will depend on the human cells collected with the specimen

One Positive and one Negative Control must be extracted and tested with each batch of patient samples that are analyzed. The expected results must be obtained with both controls to enable reporting of patient results.

## H. INTERPRETATION OF RESULTS

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted (Refer to Table 6 for a summary of control results).

**Table 3: Interpretation of results for quality controls**

| Quality control metrics | VIC (β-actin) | FAM (SARS-CoV-2) | Interpretation |
|---|---|---|---|
| Negative Control | No amplification | No amplification | Pass; proceed to sample analysis |
| Positive Control | Sigmoidal amplification curve; Ct <35 | Sigmoidal amplification curve; Ct <37 | |
| Negative Control | Sigmoidal amplification curve; Ct <35 | Sigmoidal amplification curve; Ct <37 | Fail; repeat run before proceeding to sample analysis |
| Positive Control | No amplification or Ct >35 | No amplification or Ct >37 | Fail; repeat run before proceeding to sample analysis |

**Table 4: Interpretation of Test Results**

| VIC (β-actin) | FAM (SARS-CoV-2) | Interpretation |
|---|---|---|
| Ct value is <35. | Ct value is <37. | **Positive for SARS-CoV-2 RNA**; amplification detected in both channels and Ct values are below the thresholds. |
| Ct value is <35. | Ct value is >37. | **Negative for SARS CoV-2 RNA**; amplification detected in both channels but Ct is above the threshold for the FAM channel. |
| Ct value is >35. | Result is inconsequential given that the internal control failed. | **Invalid - repeat test**; [1] amplification detected in the VIC channel but Ct is above the threshold. |
| Ct value is >35. | Ct value is >37. | **Invalid - repeat test**; [1] Ct values for the VIC and FAM channels are above the thresholds. |

[1] Repeat the test with by re-extracting RNA from the original specimen. If this test also fails, then collect a new specimen from the patient and repeat the test.

## LIMITATIONS

The use of this assay as an *in vitro* diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests. Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

The performance of UDX SARS-CoV-2 Molecular Assay was established using nasopharyngeal swabs. Nasal swabs, mid-turbinate nasal swabs and oropharyngeal swabs are also considered acceptable specimen types for use with the UDX SARS-CoV-2 Molecular Assay but performance has not been established. Testing of nasal and mid-turbinate nasal swabs (self- collected at a healthcare site or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information. Samples must be collected, transported, and stored

using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences. Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

False-negative results may arise from:

- Improper sample collection
- Degradation of the viral RNA during shipping/storage
- Using unauthorized extraction or assay reagents
- The presence of RT-PCR inhibitors
- Mutation in the SARS-CoV-2 virus
    - Failure to follow instructions for use

False-positive results may arise from:

- Cross contamination during specimen handling or preparation
- Cross contamination between patient samples
- Specimen mix-up
- RNA contamination during product handling

The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not yet been evaluated.

Please note, Negative results do not preclude infection of SARS-CoV-2 virus and should not be the sole basis of a patient management decision. A positive result indicates detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable. Laboratories are required to report all positive results to the appropriate public health authorities

## PERFORMANCE EVALUATION

### 1) ***Analytical Sensitivity:***

The Limit of Detection (LOD) was determined for the UDX SARS-CoV-2 Molecular Assay. The Limit of Detection is the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all replicates test positive. Nasopharyngeal swabs were collected from individuals negative for SARS-CoV-2 and placed into Universal Viral Transport Media and ThinPrep (SDI swab). Synthetic SARS-CoV-2 viral RNA was diluted into the sample matrix in dilutions/concentration of 10,000; 5,000; 2500; 1000; 500; 250; 100 and 50 copies/mL. Each concentration was tested with three replicates. The samples

were extracted on the procedure KingFisher Flex Purification System and then RT-PCR was performed on the QuantStudio 12K platform.

To confirm the Limit of Detection, 20 replicates at the lowest level detected were tested: 100 copies/mL. Nasopharyngeal swabs were collected from individuals negative for SARS-CoV-2 and placed into Universal Viral Transport Media. Synthetic SARS-CoV-2 viral RNA target sequence was diluted into the sample matrix was diluted at the lowest level detected above. The samples were extracted on the KingFisher Flex Purification System and then RT-PCR was performed on the QuantStudio.

**Table 5: LoD Study Confirmation Summary**

| Target | SARS-CoV-2 target in NP (UTM) | SARS-CoV-2 target in NP (ThinPrep) |
|---|---|---|
| RNA Concentration | 100 copies/mL | 100 copies/mL |
| Positives/Total | 20/20 | 20/20 |
| Mean Ct | 33.42 | 36.67 |
| SD (Ct) | 0.65 | 0.85 |
| CV | 1.94% | 2.32% |

The LoD was confirmed to be 100 copies/mL based on a positivity rate of ≥95% for 20 replicates.

***2) Analytical Inclusivity:***

*In silico* analysis was conducted for SARS-CoV-2 strains. Inclusivity is defined as 100% homology between 'primer set' and any sequence present in the targeted microorganism. The gene amplicons for each target were tested on the Basic Local Alignment Search Tool (BLAST) located on the National Center for Biotechnology Information (NCBI) a division of the National Institutes of Health (NIH) (https://blast.ncbi.nlm.nih.gov/Blast.cgi ) against 284 publicly available SARS-CoV-2 sequences on March 10, 2020. The Primer NPC1-YF22 and probe NPC1-P2 exhibited 100% homology with all the available sequences.

***3) Cross-Reactivity:***

*In silico* analysis of the UDX SARS-CoV-2 Molecular Assay primers and probes against the sequences of 47 different pathogens demonstrated no evidence of the potential for cross-reaction or interference. Although individual primers exhibited ≥80% homology with sequences from some pathogens, including Adenovirus, *Bacillus* spp., *Bacteroidetes*, and Influenza A, the potential for exponential amplification/detection that could result in cross-reaction and/or interference was determined to be low.

The analytical specificity of the UDX SARS-CoV-2 Molecular Assay was evaluated by wet testing of other organisms and viruses that may be present in respiratory specimens. Zeptometrix validation controls containing most common respiratory viruses and bacteria as recommended by the FDA (in concentration ranging from 10,000 to 100,000 copies/mL) were extracted individually as patient specimens and then and tested for SARS-CoV-2 for specificity and cross-reactivity.

**Table 6: Cross-Reactivity Wet-Testing**

| Sequence | Microorganisms/Pathogen | Strain/species | Results | Ct | Expected |
|---|---|---|---|---|---|
| MH1 | Influenza A H1N1 | A/New Caledonia/20/99 | Not Detected | 32.26 | Not Detected |
| MH2 | Influenza A H3 | A/Brisbane/10/07 | Not Detected | 0.00 | Not Detected |
| MH3 | Influenza A 2009 H1N1pdm | A/NY/02/09** | Not Detected | 0.00 | Not Detected |
| MH4 | Influenza B | B/Florida/02/06 | Not Detected | 0.00 | Not Detected |
| MH5 | Metapneumovirus 8*** | Peru6-2003 | Not Detected | 0.00 | Not Detected |
| MH6 | Respiratory Syncytial virus A | N/A | Not Detected | 0.00 | Not Detected |
| MH7 | Rhinovirus 1A | N/A | Not Detected | 0.00 | Not Detected |
| MH8 | Parainfluenza virus Type 1 | N/A | Not Detected | 0.00 | Not Detected |
| MH9 | Parainfluenza virus Type 2 | N/A | Not Detected | 0.00 | Not Detected |
| MH10 | Parainfluenza virus Type 3 | N/A | Not Detected | 34.12 | Not Detected |
| MH11 | Parainfluenza virus Type 4 | N/A | Not Detected | 0.00 | Not Detected |
| MH12 | Adenovirus Type 3 | N/A | Not Detected | 0.00 | Not Detected |
| MH13 | Coronavirus NL63 | N/A | Not Detected | 0.00 | Not Detected |
| MH14 | Coronavirus 229E | N/A | Not Detected | 0.00 | Not Detected |
| MH15 | Coronavirus OC43 | N/A | Not Detected | 0.00 | Not Detected |
| MH16 | Coronavirus HKU-1 | N/A | Not Detected | 0.00 | Not Detected |
| MH17 | M. pneumoniae | M129 | Not Detected | 0.00 | Not Detected |
| MH18 | C. pneumoniae | CWL-029 | Not Detected | 0.00 | Not Detected |
| MH19 | B. pertussis | A639 | Not Detected | 0.00 | Not Detected |
| MH20 | Adenovirus Type 31 | N/A | Not Detected | 0.00 | Not Detected |
| MH21 | Adenovirus Type 1 | N/A | Not Detected | 0.00 | Not Detected |
| MH22 | B. parapertussis | A747 | Not Detected | 38.10 | Not Detected |
| MH23 | Negative | NA | Not Detected | 0 | Not Detected |

## *4) Clinical Evaluation*

The performance of the UDX SARS-CoV-2 Molecular Assay was evaluated with blind contrived samples. All samples were prepared with nasopharyngeal (NP) swabs from individuals negative for SARS-CoV-2 collected in Universal Viral Transport Media and ThinPrep. Positive samples were contrived by adding Synthetic SARS-CoV-2 viral RNA

pseudovirus. The samples were extracted on the KingFisher Flex Purification System and then RT-PCR was performed on the QuantStudio. A total of 120 samples were tested including 60 negative samples and 60 positive samples (30 NP swabs in UTM and 30 NP swabs in ThinPrep) spanning various SARS-CoV-2 RNA concentrations. A summary of results is provided in the Table below.

**Table 7: Clinical Evaluation Analyzed Results**

| Sample | Conc. (RNA copies/mL) | Number of Samples | Number Positive^ | % Performance Agreement | 95 % CI |
|---|---|---|---|---|---|
| Negative | 0 | 60 | 0 | 100% | 94-100% |
| LOD | 100 | 20 | 20 | 100% | 83.9-100% |
| 10x LOD | 1,000 | 20 | 20 | 100% | 83.9-100% |
| 100x LOD | 10,000 | 20 | 20 | 100% | 83.9-100% |

The results of 19 positive and 21 negative specimens tested with the SDI LAB SARS-COV-2 ASSAY (EUA200122) at Specialty Diagnostics Laboratory was confirmed using an alternative assay and fulfills the requirement for confirmatory testing of at least five positive and five negative specimens.